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A 13-Year Retrospective Study on Primary Liver Cancer in Cambodia: A Strikingly High Hepatitis C Occurrence among Hepatocellular Carcinoma Cases

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Key Words
Hepatocellular carcinoma · Cholangiocarcinoma · Hepatitis B virus · Hepatitis C virus · *Opisthorchis viverrini* · Cambodia

Abstract
Objectives: Hepatocellular carcinoma (HCC) is the main type of primary liver cancer (PLC) worldwide, but cholangiocarcinoma (CCA) may be predominant in some specific regions of Southeast Asia. The aim of the present study was to delineate a pattern of Cambodian PLC patients attending the Calmette Hospital in the Cambodian capital Phnom Penh. ***Materials and Methods:*** A total of 553 medical charts diagnosing PLCs from January 2003 to May 2015 were obtained from both the Oncology and Hepato-Gastroenterology Departments of the Calmette Hospital. ***Results:*** HCC was the predominant type of PLC recorded, with 511 cases (92.4%), whereas CCA represented merely 7.6% (42 cases) of the overall series. Hepatitis B virus (HBV; 44.3%) and hepatitis C virus (HCV; 43%) infection rates were similar among the HCC patients, while small subsets of CCA patients were infected with HBV (15.4%) or HCV (11.5%). Most HCC (84%) and CCA (73.8%) patients received palliative treatment only. ***Conclusion:*** The present study indicates that HCC is the main form of primary hepatic neoplasm among PLC patients attending a hospital in Cambodia. HBV and HCV infections represented equivalent burdens and major contributing factors to HCC. Therefore, the implementation of prevention programs for these infectious agents should become a priority for health policy makers in the country.

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The work was conducted in the Calmette Hospital, Phnom Penh, Cambodia.

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Introduction

Primary liver cancer (PLC) is associated with a high mortality rate; it is the second cause of cancer-related death worldwide [1]. Along with Sub-Saharan Africa, Eastern and Southeastern Asia represents a major region of incidence of the disease, ranking respectively first and second, both in terms of incidence and mortality [2]. In Cambodia, very little data about the cancer burden is currently available. According to the estimates available, 20% of all male cancers in the country are PLCs, making it the most frequent tumor in Cambodian men [3, 4].

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are the two main histologic types of PLC [5]. HCC is globally known as the leading form of liver cancer and accounts for up to 85% of incidences worldwide [6]. However, CCA represents the predominant form of PLC in some specific regions of Southeast Asia. For instance, CCA is responsible for 90% of the liver cancer cases monitored in the Khon Kaen province of Thailand [7–9].

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are considered to be the main etiological factors for HCC. These two infectious agents are estimated to be responsible for more than three quarters (78%) of the HCC cases worldwide [10]. In Southeast Asia, which is a highly endemic area for HBV, the major risk factor for HCC is chronic HBV infection [2, 11]. So far, the number of cases of HCC related to HCV infection in the region was poorly known and considered to be of marginal significance when compared with that linked to HBV [10]. Concerning the risk factor for CCA, it results largely from the infection with the liver fluke *Opisthorchis viverrini* endemic to Cambodia, Laos, Thailand, and Vietnam [8, 9, 12]. According to the International Agency for Research on Cancer (IARC), 6 million people are infected with *O. viverrini* in Thailand, 2 million in Laos, and 600,000 in Cambodia [13].

In Cambodia, hepatitis B surface antigen (HBsAg) seroprevalence in the general population is estimated at between 7.7 and 13%, whereas the hepatitis C antibody seroprevalence range is between 2.3 and 14.7% [4, 14–18]. Concerning the liver fluke, a high parasitic prevalence has been recorded in some provinces, especially in Kampong Cham, Kampong Thom, Kandal, Kratie, and Takeo provinces. In these regions, infestation rates among the general population range from 1.3 to 65.1% [19–22].

With the exception of a single study that aimed to describe the clinical and demographic features of Cambodian HCC patients at a single center [4], no data have been published on CCA and HCC tumor presentation in this country. Therefore, the present work was carried out with the objective of describing and comparing the clinico-epidemiological features of both CCA and HCC in Cambodian patients attending the Calmette Hospital (Phnom Penh, Cambodia) over a 13-year period.

Materials and Methods

The Department of Oncology of the Calmette Hospital, was opened in 2012. Prior to 2012, the Department of Hepato-Gastroenterology was in charge of attending to the PLC patients. We thus carried out a retrospective study of the archives of these two departments in order to collate the PLC medical charts that had been compiled over an almost 13-year period from January 2003 to May 2015.

A total of 553 medical charts of PLC patients were reviewed. The data provided on paper, such as medical reports, daily follow-up sheets, laboratory and imaging results, were extracted from the patients' files available in each department and recorded in Excel file version 2013 (Microsoft). Sociodemographic information, clinical manifestations, biological analyses, medical imaging, method of diagnosis, and treatment outcomes were also monitored and compiled into the database.

However, due to poor resources in the country, none of the files examined included all items listed above. As a consequence, serological parameters, biochemistry analyses, diagnostic imaging, and therapeutic options were not always available (ranging from 60% for the γ-glutamyl transpeptidase level to 100% for clinical features).

The diagnosis of HCC at the Calmette Hospital was based on clinical, biochemical, and imaging criteria. The three decisive features were: (1) the presence of liver cirrhosis and signs of neoplastic disease in an alcoholic patient or a patient chronically infected with HBV or HCV, (2) α-fetoprotein (AFP) >350 ng/ml, and (3) nodule size over 20 mm (by ultrasound examination). In other cases (AFP <350 ng/ml and/or nodule size ranging between 10 and 20 mm), the diagnosis was made using computed tomography showing a typical arterial pattern. Regarding clinical symptoms, ascites, portal hypertension, jaundice, liver mass at palpation, hepatomegaly, liver collateral circulation, splenomegaly, hemorrhages, bruises, encephalopathy, pain, and weight loss were the most frequent features found in patients. In the case of CCA patients, the diagnosis was predominantly made based on personal background (presence of primary sclerosing cholangitis), clinical features (jaundice, pale stool, dark urine, and pruritus), biochemical parameters (elevation in total bilirubin, direct bilirubin, and aminotransferases) suggesting a biliary obstruction, the presence of tumor markers (elevation in CEA and CA 19-9, and normal AFP) as well as on ultrasound examination (biliary ductal dilatation and related mass lesion) and on the results of the CT scan (bile duct dilatation and tumor mass).

Differential diagnosis between HCC and CCA was based on several criteria: personal background and risk factors (chronic HBV/HCV infection, alcohol consumption), clinical features (liver cirrhosis, signs of portal hypertension, signs of biliary obstruction, ascites), laboratory parameters (transaminases, bilirubin, AFP, CEA, CA 19-9), and imaging techniques (location and size of the mass, enhancement pattern).

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Table 1. Characteristics of the HCC and CCA patients

Patients characteristics	HCC patients	CCA patients
Overall subjects		
Total number	511 (92.4%)	42 (7.6%)
Age (n = 511/42), years		
Mean ± SD	58.1±11.9	62.3±11.9
Range	28–91	40–90
Sex (n = 511/42)		
Male	369	21
Female	142	21
Sex ratio	2.6	1
Symptoms and signs (n = 511/42)		
Abdominal pain	263 (51.4%)	25 (59.5%)
Ascites	340 (66.5%)	12 (28.6%)
Asthenia	132 (25.8%)	9 (21.4%)
Fever	69 (13.5%)	7 (16.7%)
Gastrointestinal bleeding	168 (32.9%)	1 (2.4%)
Hepatomegaly	226 (44.2%)	9 (21.4%)
Jaundice	131 (25.6%)	31 (73.8%)
Edema	37 (7.2%)	ND
Pruritus	6 (1.2%)	11 (26.2%)
Splenomegaly	278 (54.4%)	5 (11.9%)
Associated disorders and risk factors		
Alcohol drinking (n = 324/30)	140 (43.2%)	4 (13.3%)
Chronic HBV infection only (n = 467/26)	207 (44.3%)	4 (15.4%)
Chronic HCV infection only (n = 467/26)	201 (43.0%)	3 (11.5%)
Chronic HBV/HCV infection (n = 467/26)	20 (4.3%)	0
Cirrhosis (n = 460/24)	454 (98.7%)	9 (37.5%)
Diabetes (n = 457/35)	90 (17.6%)	4 (11.4%)
Hypertension (n = 456/35)	102 (20.0%)	7 (20.0%)

Unless otherwise indicated the values represent the number. Mean values are associated with the standard deviation (SD). The sex ratio was calculated as males:females. n = Total number of HCC patients/CCA patients with data available; ND = not determined.

Imaging modalities included mostly ultrasonography testing for 94.3% HCC patients and 94.1% CCA patients, while only 15.2% HCC patients and 58.8% CCA patients had a computed tomography scan. Neither liver biopsy nor magnetic resonance imaging was performed.

Regarding the clinical chemistry, laboratory analyses were performed in the Biochemistry Department of the Calmette Hospital, where the following parameters were determined: γ-glutamyl transpeptidase, alkaline phosphatase, aspartate and alanine aminotransferase, bilirubin, prothrombin time, hemoglobin, albumin, and AFP. It should be noted that, until 2014, the AFP level was indicated as '>350 ng/ml' for each value above 350 ng/ml in the biological report. Thus, no fully accurate data were available for this parameter.

Other information, which would have been useful for the study, including tumor grade, infection by *O. viverrini*, aflatoxin contamination, arsenic exposure, type of alcohol consumed, pack-year units for cigarette smoking, and associated disorders (except diabetes and hypertension), was not present in the medical records. Moreover, no precise information was given for the serological tests used in the diagnosis of HBV and HCV.

Different statistical analyses were performed according to the set of data analyzed. Data were recorded in Microsoft Excel 2013 (Microsoft) and later analyzed using SPSS Statistic software version 22.0 (IBM). Comparisons between groups (numerical data) or proportions (categorical data) were realized using the Student t test or Mann-Whitney test, ANOVA and the Fisher exact test as appropriate. Results of the analysis were considered statistically significant if the probability of occurrence by chance was less than 5%. The free and open-source QGIS software version 2.8.2 was used to elaborate repartition maps.

The study was carried out in accordance with the ethical principles of the Declaration of Helsinki and was approved by the National Ethics Committee for Health Research under the guidance of the Ministry of Health of Cambodia in January 2015.

Results

Descriptive Characteristics of the 553 Patients

Out of the 553 patients included, 511 (92.4%) had a diagnosis of HCC and 42 (7.6%) were diagnosed with CCA. The demographic characteristics of HCC and CCA

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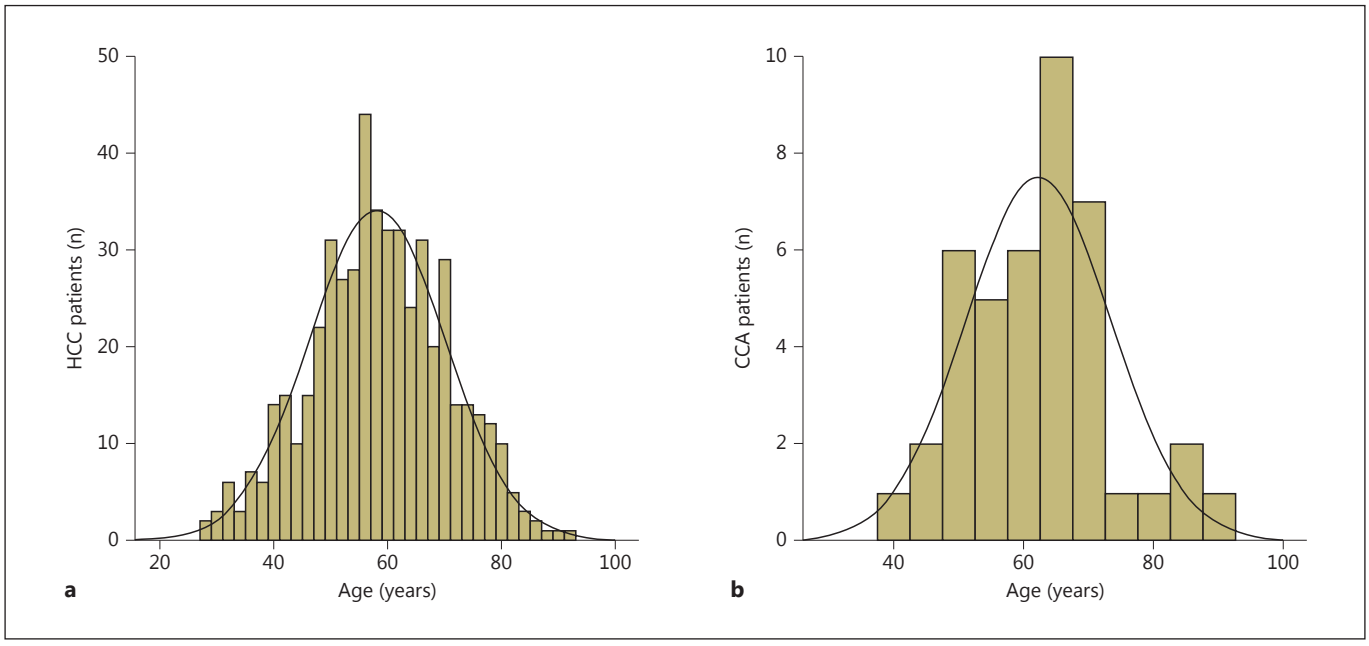

Fig. 1. Age distribution of the 511 HCC (**a**) and 42 CCA (**b**) patients admitted to the Calmette Hospital between 2003 and 2015.

patients as well as the clinical manifestations, risk factors, and associated disorders are given in table 1.

Of the HCC patients, a large majority (76.9%) were more than 50 years old. The distribution of the data was fairly symmetrical (i.e., skewness coefficient = –0.04). Of the 42 CCA patients, 81% were more than 50 years old (fig. 1).

A large subset of the HCC patients was farmers (n = 136; 33.6%), whereas a majority of CCA patients were housewives or unemployed (n = 14; 40%). Professions of the remaining HCC cases were, in decreasing order, either housewives/unemployed (n = 98; 24.2%), public sector employees (n = 87; 21.5%), or private sector employees (n = 84; 20.7%). Regarding CCA cases, 10 (28.6%) were farmers, 7 (20%) were private sector employees, and 4 (11.4%) were public sector employees. Following hospitalization, the average length of the medical stay was 6.5 and 5.9 days for HCC and CCA patients, respectively.

As mentioned above, information was not always available for the entire series. As a consequence, we mention the true numbers corresponding to each parameter, which is the total number of HCC or CCA cases with data recorded (tables 1, 2).

Interestingly, chronic HBV and HCV infections (i.e., HBsAg+ and anti-HCV+, respectively) showed similar rates in the HCC patient population, with 207 (44.3%) HBsAg+ cases and 201 (43%) anti-HCV+ cases. Moreover, 20 patients (4.3%) were infected both with HBV and HCV, while only 38 patients (8.1%) were neither infected with HBV nor with HCV.

The mean age of HBsAg+ and anti-HCV– patients (n = 207) was 52.1 ± 12.0 years, with an age range of 28–91 years. The mean age of HBsAg– and anti-HCV+ patients (n = 201) was 63.9 ± 12.0 years, with a range of 36–89 years.

Amongst HCC patients under 50 years, 99 (73.9%) were infected only with HBV (HBAgs+/anti-HCV–), whereas only 12 (9%) were infected with HCV alone (HBAgs–/anti-HCV+). Reciprocally, among the 229 HCC cases aged over 60 years, 53 (23.1%) were infected with HBV, and 133 (58.1%) were infected with HCV.

In CCA patients, serological results were available for only 26 patients. Among these, 19 (73.1%) were neither infected with HBV nor with HCV, 4 (15.4%) had chronic HBV infection, and 3 (11.5%) had chronic HCV infection.

As shown in table 2, an elevation of serum aspartate aminotransferase and alanine aminotransferase was found, respectively, in 98 and 61% of the HCC cases and in 89.7 and 46.2% for the CCA cases. Total and direct bilirubin levels were increased in 84.8 and 88.6% of the HCC cases and in 89.5 and 97.4% of the CCA patients. γ-Glutamyl transpeptidase and alkaline phosphatase levels were elevated for 82.3 and 73.2% of the HCC patients,

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Table 2. Laboratory and radiologic findings of the HCC and CCA patients

Patient characteristics	HCC patients	CCA patients
Liver function test		
AST (R <38) (n = 461/39), IU/l	196.8 (14–3,359)	124.6 (24–351)
ALT (R <50) (n = 461/39), IU/l	77.1 (10–415)	69.1 (13–102)
Total bilirubin (R <10) (n = 446/38), mg/l	61.6 (4–1,069)	183.5 (6–485)
Direct bilirubin (R <2.5) (n = 446/38), mg/l	36.4 (1–897)	123.3 (2–401)
GGT (R <66) (n = 344/32), IU/l	246.8 (12–3,412)	284.7 (11–1,213)
ALP (35< R <123) (n = 306/29), IU/l	278.6 (36–1,928)	431.2 (85–1,152)
Prothrombin time (70< R <100) (n = 420/28), %	60.4 (5–100)	71.8 (26–100)
Hemoglobin (130< R <170) (n = 366/16), g/l	101.7 (25–171)	92.3 (32–139)
Albumin (38< R <51) (n = 392/23), g/l	28.8 (15–52)	29.3 (20–48)
AFP (R <10) (n = 366/19)		
<10 μg/l	53 (14.5%)	16 (84.2%)
10–100 μg/l	50 (13.7%)	2 (10.5%)
100–350 μg/l	32 (8.7%)	0
>350 μg/l	231 (63.1%)	1 (5.3%)
Imaging features		
Nodule size (n = 345/13), mm	64.1 (10–185)	39.8 (22–72)
Multinodular type (n = 319/15)	212 (66.5%)	6 (40%)
Therapy (n = 511/42)		
Palliative	429 (84%)	31 (73.8%)
Systemic/oral chemotherapy	71 (13.9%)	11 (26.2%)
Hepatectomy	11 (2.1%)	0
PEI/TOEC	0	0
Transplantation	0	0

Values represent mean (range) or number. n = Total number of HCC patients/CCA patients with data available; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = γ-glutamyl transpeptidase; PEI = percutaneous ethanol injection; R= reference values; TOEC = transarterial oil chemoembolization.

respectively, and for 90.6 and 93.1% of the CCA patients. Statistical analyses of biochemistry parameters were also performed (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000446398).

The AFP level was documented for 366 HCC patients and 19 CCA patients, with the corresponding percentage of 63.1 and 5.3% values above 350 ng/ml. High AFP levels (>350 ng/ml) among HCC patients are associated significantly with larger nodules (p = 0.05) and multifocal tumor (p = 0.03) when compared with low AFP expressers (<350 ng/ml). Moreover, statistical analyses between a normal AFP level (<10 ng/ml) and an abnormal AFP level (>10 ng/ml) in HCC patients indicate a significant association with the latter and chronic HCV infection (p = 0.05).

Palliative treatment was the only option for 429 (84%) HCC patients; only 11 (2.1%) patients had a hepatectomy, 10 (2.0%) patients were given oral chemotherapy with sorafenib, and 61 (11.9%) patients received tamoxifen. Of the CCA patients, 11 (26.2%) benefited from systemic chemotherapy (including GEMOX, FOLFOX, and FOLFIRI protocols), whereas 31 patients (73.8%) received palliative treatment only. Some of the most affluent patients decided, however, to benefit from surgical intervention outside the country. They represent approximately 5–10% of the patients attending the Calmette Hospital for liver tumor.

Clinicobiological Correlations

Age was an important feature structuring the series of patients with HCC. When considering the whole cohort in age quartiles (fig. 2a–e), significant differences related to age were found for HBsAg seroreactivity (p < 0.0001), sex ratio (p = 0.0014), nodule size (p = 0.0016), and alcohol consumption (p < 0.0001) that decrease with age, while the proportion of diabetes (p = 0.0005) and the anti-HCV seroreactivity increase with age (p < 0.0001). Finally, the prevalence of abdominal pain (p = 0.0008) and jaundice decrease with age (p = 0.008).

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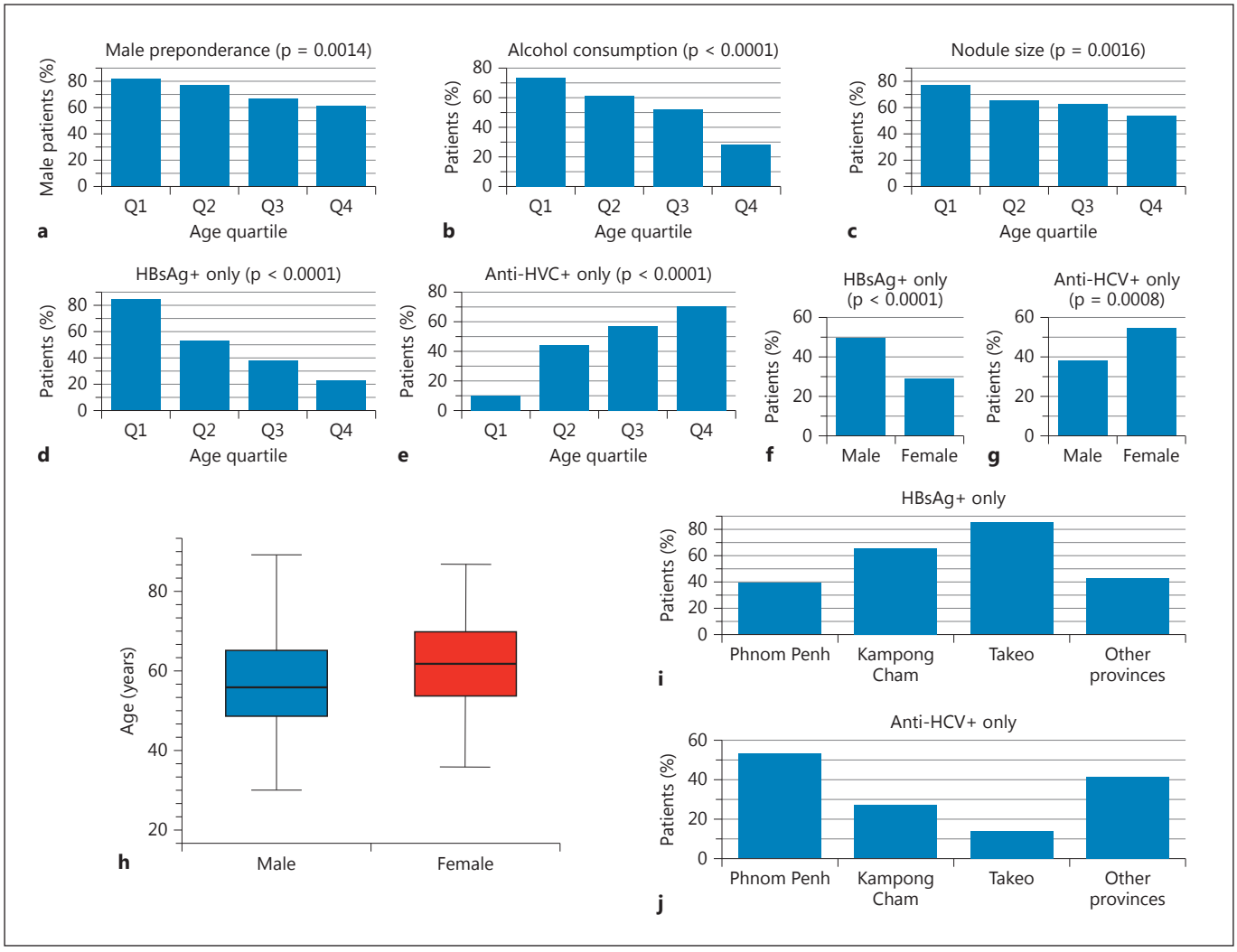

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Fig. 2. Age quartile comparisons among HCC patients according to male preponderance (**a**), alcohol consumption (**b**), nodule size (**c**), HBsAg+ only (**d**), and anti-HCV only (**e**). Q1 = Quartile 1 (28–49 years old); Q2 = quartile 2 (50–57 years old); Q3 = quartile 3 (58–65 years old); Q4 = quartile 4 (66–91 years old). Gender comparisons among HCC patients: percentage of males and females with HBsAg+ only (**f**) and anti-HCV+ only (**g**). **h** Box plot representing age distribution in male (blue) and female (red) HCC patients (colors in the online version only). Geographical comparisons among HCC patients: bar charts displaying the percentage of HBV- (**i**) and HCV- (**j**) infected patients with HCC according to their province of residence.

In addition, we conducted gender comparisons amongst HCC patients (fig. 2f–h). The results indicate that men develop tumors 3 years earlier than women (56.8 vs. 61.5 years; $p < 0.0001$), hepatomegaly prevalence was higher in men (48.8 vs. 32.4%; $p = 0.001$), while arterial hypertension was more frequent among women (16.8 vs. 36.7%; $p < 0.0001$). Regarding lifestyle risk factors, alcohol consumption and tobacco smoking were, as expected, significantly higher in men than in women (50.6 vs. 17.9% and 48.9 vs. 5.6%; $p < 0.0001$ in both cases), whereas anti-HCV seroreactivity was more frequent in the female group (38.1 vs. 55.4%; $p = 0.0009$). Symmetrically, HBsAg seropositivity was more prevalent among males (50 vs. 29.4%; $p < 0.0001$).

We then compared the two monoinfected subsets of patients. The results show that the sex ratio was significantly higher in the HBV+ group than in the HCV+ group (4.4 vs. 1.8; $p < 0.0001$), the patients were younger (52.1 vs. 63.8 years; $p < 0.0001$), and alcohol consumption was more frequent in the HBV+ group (45.7 vs. 32.5%; $p = 0.04$), whereas diabetes mellitus was less frequent (15.5 vs. 25.6%; $p = 0.02$). Regarding biochemical features, serum

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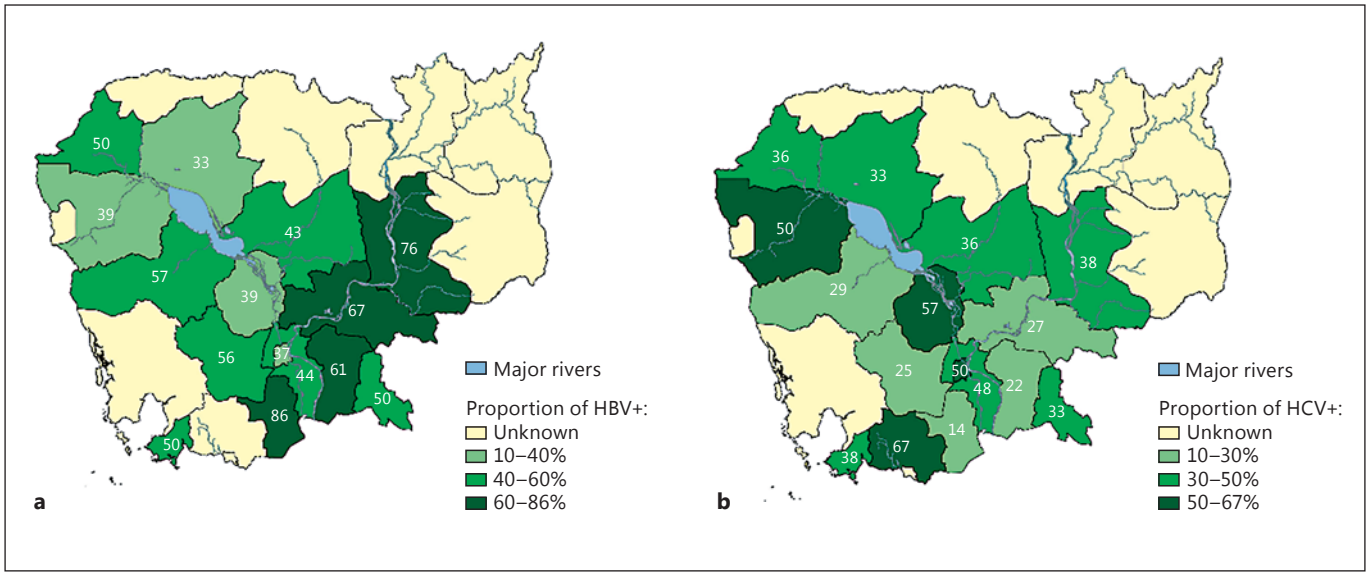

Fig. 3. Regional distribution of HBV- (**a**) and HCV- (**b**) infected patients with HCC attending the Calmette Hospital between 2003 and 2015. Numbers on the map indicate the percentage of HBV-HCC (**a**) and HCV-HCC (**b**) patients.

alkaline phosphatase and serum aspartate aminotransferase were significantly higher (302.8 vs. 218.7 and 213.5 vs. 170.4 IU/l; $p < 0.05$ in both cases), and tumor nodules were bigger in the HBV+ group (67.5 vs. 59.3 mm; $p = 0.04$) compared to the HCV+ group.

Frequency and Spatial Distribution of HCC and CCA Patients

Among the HCC patient population, the highest number of cases originated from the Phnom Penh province (n = 197; 38.6%) followed by the Kandal province (n = 59; 11.5%) and the Kampong Cham province (n = 46; 9%). In the case of CCA, Phnom Penh (n = 13; 30.9%), Kampong Cham, and Kandal provinces (both n = 6; 14.3%) exhibited the highest values (online suppl. fig. 1).

Concerning the characteristics of HCC patients for each province, we noticed that Phnom Penh and Kandal provinces display similar features, such as an older age of patients (a mean of 60.8 and 59.4 years, respectively), a male:female sex ratio of 2.5 and 2.3, respectively, and a high proportion of diabetes mellitus (30.2 and 25.9%, respectively). Conversely, patients from the Prey Veng and Takeo provinces, on the southern border, share comparable features of young age at diagnosis (a mean of 52.8 and 51.4 years, respectively), an elevated sex ratio (5.6 and 14, respectively), a high proportion of alcohol consumers (53 and 33%, respectively), and a high number of HBsAg+ cases (61 and 86%, respectively). Concerning the spatial distribution of HBsAg+ and anti-HCV+ HCC patients, we observed a great disparity of distribution of the two types of hepatitis viruses between different Cambodian provinces (fig. 2i, j, 3).

In Phnom Penh, the proportion of patients infected with HBV was significantly lower than in other provinces (39.8 vs. 50.5%, respectively, $p = 0.03$), whilst, in contrast, a significantly greater subset was infected with HCV (53.2 vs. 39.4%, respectively, $p = 0.004$). Similarly, Kampong Cham province displayed a strikingly different proportion of patients infected either with HBV or with HCV (66.7 and 27.3%, respectively) with significant differences in both subpopulations ($p = 0.006$ and $p = 0.02$, respectively). Finally, Takeo province showed the highest proportion of HBV+ patients (85.8%, $p = 0.004$) and the lowest proportion of HCV+ patients (14.3%, $p = 0.03$).

Discussion

This study aimed to characterize the forms and the etiological factors of PLC in Cambodia through a retrospective analysis of the cohort treated in the Calmette Hospital from 2003 and 2015. It is also the first review on CCA patients in the country and provides relevant information

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about epidemiological features of HCC and CCA among Cambodian people.

Interestingly, we found a similar rate of chronic infection with HBV (44.3%) and HCV (43%) among HCC patients. This observation was unexpected, since the overall contribution of HBV infection to PLC is more than twice higher than that of HCV infection worldwide, 53 and 25%, respectively [10].

However, the infection rate found in our study cannot be extrapolated to the whole country, since the only available survey of PLC cases in Cambodia showed that the HBV and the HCV infection rate in HCC patients was 46.4 and 21.5%, respectively [4]. In this earlier study, the demographic and clinical characteristics of 281 cases diagnosed as primary HCC in the Khmer-Soviet Friendship Hospital (Phnom Penh, Cambodia) were described. The majority of the HCC patients were aged between 40 and 69 (74.4%) and the male:female ratio was 3. These results are consistent with our data, since we found that 74.3% of the HCC cases were aged between 40 and 69 years, and the male:female ratio was 2.6. Furthermore, the mean age of the HCV+ HCC cases was 10 years higher than those with HBV+ (61.4 vs. 48 years old), which is quite similar to our results (63.9 vs. 52.1 years old). Moreover, the male:female ratio for HBV+ HCC patients was also similar to that found in our survey (4.4 and 4.5, respectively). Finally, the male:female ratio for HCV+ HCC cases was different to that found in our study (4 and 1.8, respectively). These data could be explained by the small number of reported cases in the Khmer-Soviet Friendship Hospital (i.e., 45 patients) as described below.

In other Southeast Asian countries, a greater proportion of HBV infections compared to HCV infections is also consistently found among HCC patients. In some regions of Thailand, chronic HBV infection was identified in 49.6–80% of HCC cases and chronic HCV infection in 10.1–19.2% [23–25]. In Vietnam, a review estimated the HCC-HBV patients at 80–90% and the HCC-HCV patients at 1–5% [26]. In Malaysia, a recent study recorded HBV in 57.6% of HCC patients and discovered HCV in only 2.4% of the same cohort [27]. In the Asia-Pacific region, chronic HBV infection accounts for 70–80% of the HCC cases, with the exception of Japan, Pakistan, and Mongolia where chronic HCV infection is predominantly found (68, 45, and 40% of the HCC cases, respectively) [23, 28].

Many studies have already reported a large local variation in the prevalence of HBV and HCV infection, especially in Southeast Asia and Cambodia. For instance, the prevalence of HBsAg was reported to be 4.6% in Siem Reap province, 6.5% in Samlot City (Battambang province), 8% in Takeo province, 8.8% in Pailin City (Pailin province), 9.4% in Banteay Meanchey province, and 10.8% in Cambodian migrant workers from Thailand [17, 18, 29–31]. For HCV infection, the prevalence of anti-HCV was 2.3% among Cambodian migrant workers from Thailand, 5.8% in Siem Reap province, 6.5% in Takeo province, 12.3% in Pailin City, and 17% in Samlot City [15, 17, 18, 29]. These observations could explain the difference between the work of Narin et al. [4] and our survey. This previous study was conducted in the Khmer-Soviet Friendship Hospital where patients are usually coming from rural areas with lower socioeconomic status, while in our study, patients are mostly capital city dwellers and belong to a higher social class.

The high number of incidences attributed to HCV in our work may appear surprising. However, it is known that there is still an increasing impact of HCV infection on the HCC burden in Asia. A recent study, conducted among Southeast Asian migrants in the US, revealed that both HBV and HCV are major causes of HCC in these populations [32]. Moreover, a recent review has identified chronic hepatitis C as the true driving force for the increased incidence of HCC in Asia [33].

In Cambodia, unsafe injection practices, the absence of quality control and recycling, and the lack of proper sterilization of surgical instruments are known to be the main risk factors for HCV contamination [34, 35]. Therefore, inappropriate health-care practices plausibly represent the predominant source of HCV contamination in the country. Much of the data we recorded support these hypotheses. In this regard, it was clearly found that women with HCC show a higher rate of hepatitis C than men. This observation has been confirmed in a recent study on Asian migrants in the US [32], showing that female gender is a risk factor for HCV-related HCC. According to Vong et al. [35], Cambodian women are more likely to receive therapeutic injections than men and are, therefore, more exposed to unsafe medical practices. Moreover, in an urban area such as Phnom Penh Province, where access to health care is easier than anywhere else in the country, patients were significantly more affected by HCV infection than HBV infection. Lastly, HCC cases with chronic HCV infection were not distributed homogenously as it is apparently the case with HBV-infected patients. These erratic contaminations could result from unsafe practices employed by local practitioners as recently shown for the 2014 HIV infection outbreak in the village of Roka (Kandal province) [36].

The clinicopathological features of HBV- and HCV-related HCC cases were drastically different. HBV-infect-

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ed patients displayed a strong male predominance, younger age, tobacco and alcohol dependency, and an aggressive tumor presentation (liver functions, frequent hepatomegaly, nodule size) whereas HCV-infected cases displayed a lower male:female ratio, an older age, and, as expected, maturity-related comorbidities (diabetes mellitus and hypertension). Similar findings have already been reported from previous studies. The age difference at diagnosis is usually explained by the natural history of infection [37–39]. Indeed, HBV contamination occurs mainly in the perinatal period or in childhood, whereas exposure to HCV usually occurs in adulthood. Moreover, the different mechanism in hepatocarcinogenesis between HBV-HCC and HCV-HCC could also explain these results.

A large majority of the PLCs recorded at the hospital was diagnosed as HCC (92.4%), while only few CCA cases were reported (7.6%). Given the high prevalence of *O. viverrini* infection (i.e., the main risk factor of CCA in the region) in some Cambodian provinces, the number of CCA patients was expected to be more important [40]. In Thailand, the country with the populations presenting the highest rates of infestation by *O. viverrini*, the proportion of diagnosed CCA cases reaches 22.1% of all PLC in Bangkok, but ranges from 4% (Songkhla province in the south) to 82.1% (Khon Kaen province near the Laotian border in the north) [5]. Several limitations of the survey such as the lack of histopathological examination could explain the low proportion of CCA diagnosed in the Calmette Hospital.

As mentioned above, our study has several limitations. First, missing data have already been reported as an important issue in Cambodian hospitals [4]. This situation is primarily due to the high cost of medical investigations, which, in the absence of health insurance, have to be paid by the patients [3]. Second, precise biological data on risk factors associated with these diseases were not obtained (i.e., *O. viverrini* infection, quantification of HBV DNA and HCV RNA, investigation of viral replication level). The third limitation of the present study stems from the fact that we studied liver cancer in a single Cambodian health-care facility, which is only partly representative of the overall HCC and CCA epidemiology of the country. Indeed, some patients are treated in private clinics and are not referred to a public hospital; others prefer to go to foreign countries (Vietnam, Thailand, and Singapore), while poor people cannot afford the cost of treatment and are either treated in a provincial hospital or not at all. Another limitation of our work is that the diagnosis was based on clinical features, tumor markers, and imaging tests. No histopathological examination was performed, and thus there might be misdiagnosis, albeit in a rather limited number of cases given the very evocative clinical context of the diseases reported (viral serology, AFP level, ultrasonography and computed tomography outcome). Finally, our survey is characterized by shortcomings inherent to cross-sectional observational studies and as such cannot compete with case-control or prospective studies. However, it has the merit to provide preliminary insights to conduct more informative epidemiological research in the future.

In conclusion, PLC represents a worrying public health issue in Cambodia. In our study, chronic HBV and HCV infections are contributing evenly to the bulk of HCC cases (44.3 and 43%, respectively), while *O. viverrini* infection is suspected to contribute to the CCA cases. The number of incidences attributed to HCV in the present series was surprisingly high (>40%) for a continental Southeast Asian country suggesting that the epidemiology of HCC has changed locally during the last decades. This result should be taken seriously, because it is reminiscent of the current ongoing HCC catastrophe in Mongolia [41]. Another observation that should stimulate the implementation of further investigations, preventive measures, and public information is the association of alcohol consumption and the early development of HCC in men. This observation is often overlooked in scientific literature and should prompt surveys investigating mutagenic/carcinogenic properties of local alcoholic beverages [42]. Our findings should now be used by local authorities in order to improve the surveillance of populations at risk to develop HCC or CCA as well as to prevent the diseases by avoidance both of transmission of the relevant infectious agents and the consumption of carcinogenic compounds.

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Erratum

In the article by Mitchell et al., entitled 'Validation of a preclinical model of diethylnitrosamine-induced hepatic neoplasia in Yucatan miniature pigs' [Oncology 2016;91:90–100, DOI: 10.1159/000446074], there is an error in the Materials and Methods section. Under the subheading of Anesthesia, the text reads:

For all procedures, pigs were anesthetized via intramuscular injection with either tiletamine or zolazepam (6 mg/kg; Zoetis, Florham Park, N.J., USA) in a mixture of ketamine hydrochloride (15 mg/kg) and acepromazine maleate (0.15 mg/kg).

However, the text should read:
For all procedures, pigs were anesthetized via intramuscular injection with tiletamine/zolazepam (6 mg/kg; Zoetis, Florham Park, N.J., USA) or a mixture of ketamine hydrochloride (15 mg/kg) and acepromazine maleate (0.15 mg/kg).

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